B. AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph on page 1 lines 5 to 9 with the following paragraph:

This application is a divisional of U.S. patent 6,800,728, patent application No.: 09/815,978, filed March 22, 2001 entitled "HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS" which claims priority to U.S. provisional patent application No. 60/191,186, filed March 22, 2000, to Schwartz, entitled "NOVEL HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS." The disclosures of the above-referenced applications are incorporated herein in their entirety.

Please replace the paragraph on page 1 lines 11 to 17 with the following paragraph:

Methods to crosslink biomolecules such as proteins, oligonucleotides and carbohydrates to each other, to radioactive and non-radioactive metal chelates, to drugs and to surfaces have allowed development of both in vitro and in vivo diagnostic assays as well as in vivo therapies. A wide variety of methods have been developed and reviewed (Greg T. Hermanson, Bioconjugate Techniques, Academic Press).

Please replace the paragraph on page 3 line 6 to page 4 line 5 with the following paragraph:

or a derivative thereof, where B is an amino or thiol reactive moiety; Y is a hydrazino group, as defined herein, an oxyamino group or a carbonyl group; and R is a divalent group having any combination of the following groups, which are combined in any order: arylene, heteroarylene, cycloalkylene, $C(R^{10})_2$, $-C(R^{10})=C(R^{10})-$, $>C=C(R^{12})(R^{13})$, $>C(R^{12})(R^{13})$, $-C \equiv C-$, O, $S(G)_a$, $P(J)_b(R^{10})$, $P(J)_b(LR^{10})$, $N(R^{10})$, $>N^+(R^{12})(R^{13})$ and C(L); where a is 0, 1 or 2; b is 0, 1, 2 or 3; G is O or NR^{10}; J is S or O; and L is S, O or NR^{10}; each R^{10} is a monovalent group independently selected from hydrogen and M^1-R^{14}; each M^1 is a divalent group independently

having any combination of the following groups, which groups are combined in any order: a direct link, arylene, heteroarylene, cycloalkylene, $C(R^{15})_2$, $-C(R^{15})=C(R^{15})-$, $>C=C(R^{12})(R^{13})$, $>C(R^{12})(R^{13})$, $-C\equiv C-$, O, $S(G)_a$, $P(J)_b(R^{15})$, $P(J)_b(L^1R^{15})$, $N(COR^{15})$ $N(R^{15})$, $>N^+(R^{12})(R^{13})$ and $C(L^1)$; where a is 0, 1 or 2; b is 0, 1, 2 or 3; G^1 is O or NR^{15}; J is S or O; and L^1 is S, O or NR^{15}; R^{14} and R^{15} are each independently selected from the group among hydrogen, halo, pseudohalo, cyano, azido, nitro, $SiR^{16}R^{17}R^{18}$, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy and $NR^{19}R^{20}$; R^{19} and R^{20} are each independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl and heterocyclyl; R^{12} and R^{13} are selected from (i) or (ii) as follows: (i) R^{12} and R^{13} are independently selected from among hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl; or (ii) R^{12} and R^{13} together form alkylene, alkenylene or cycloalkylene; R^{16}, R^{17} and R^{18} are each independently a monovalent group selected from hydrogen, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy and $NR^{19}R^{20}$; and

Please replace the paragraph on page 4 lines 6 to 22 with the following paragraph:

R^{10}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{20} can be substituted with one or more substituents each independently selected from Z, wherein Z is selected from alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, hydroxy, $S(O)_hR^{30}$, $NR^{30}R^{31}$, $COOR^{30}$, COR^{30}, $CONR^{30}R^{31}$, $OC(O)NR^{30}R^{31}$, $N(R^{30})C(O)R^{31}$, alkoxy, aryloxy, heteroaryl, heterocyclyl, heteroaryloxy, heterocyclyloxy, aralkyl, aralkenyl, aralkynyl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, aralkoxy, heteroaralkoxy,

alkoxycarbonyl, carbamoyl, thiocarbamoyl, alkoxycarbonyl, carboxyaryl, halo, pseudohalo, haloalkyl and carboxamido; h is 0, 1 or 2; and R^{30} and R^{31} are each independently selected from among hydrogen, halo, pseudohalo, cyano, azido, nitro, trialkylsilyl, dialkylarylsilyl, alkyldiarylsilyl, triarylsilyl, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy, amino, amido, alkylamino, dialkylamino, alkylarylamino, diarylamino and arylamino.

Please replace the paragraph on page 4 line 23 to page 5 line 1 with the following paragraph:

Thus, the reagents provided herein are aliphatic and aromatic crosslinking compounds that possess (i) a thiol or amine reactive group; and (ii) a hydrazino, oxyamino or carbonyl group. Thiol reactive groups are moieties that react directly with sulfhydryl groups forming stable thioether bonds. These thiol reactive groups include, but are not limited to, maleimido, α-bromoacetamido and pyridyldisulfides. Amino reactive moieties are those that react directly with amine moieties forming amide bonds. These amino reactive groups include, but are not limited to, N-hydroxysuccinimidyl, p-nitrophenyl, pentafluorophenyl and N-hydroxybenzotriazolyl esters.

Please replace the paragraph on page 9 line 26 to page 10 line 24 with the following paragraph:

As used herein, a derivative of a compound is a salt, ester, enol ether, enol ester, solvate or hydrate thereof that can be prepared by those of skill in this art using known methods for such derivatization. Salts are amine salts, such as but not limited to N,N'-dibenzylethylenediamine, chloroprocaine, choline, ammonia,

diethanolamine and other hydroxyalkylamines, ethylenediamine, N-methylglucamine, procaine, N-benzylphenethylamine, 1-para-chlorobenzyl-2-pyrrolidin-1'-ylmethylbenzimidazole, diethylamine and other alkylamines, piperazine and tris(hydroxymethyl)aminomethane; alkali metal salts, such as but not limited to lithium, potassium and sodium; alkali earth metal salts, such as but not limited to barium, calcium and magnesium; transition metal salts, such as but not limited to zinc; and other metal salts, such as but not limited to sodium hydrogen phosphate and disodium phosphate; and also, but not limited to, salts of mineral acids, such as but not limited to hydrochlorides and sulfates; and salts of organic acids, such as but not limited to acetates, lactates, malates, tartrates, citrates, ascorbates, succinates, butyrates, valerates and fumarates. Esters are, but are not limited to, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl and heterocyclyl esters of acidic groups, such as, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids and boronic acids. Enol ethers are, but are not limited to, derivatives of formula C=C(OR) where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl ar heterocyclyl. Enol esters are, but are not limited to, derivatives of formula C=C(OC(O)R) where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl ar heterocyclyl. Solvates and hydrates are complexes of a compound with one or more solvent or water molecule, preferably 1 to about 100, more preferably 1 to about 10, most preferably one to about 2, 3 or 4, solvent or water molecules.

Please replace the paragraph on page 11 line 1 to 16 with the following paragraph:

As used herein, alkyl, alkenyl and alkynyl carbon chains, if not specified, contain from 1 to 20 carbons, preferably 1 to 16 carbons, and are straight or branched. Alkenyl carbon chains of from 2 to 20 carbons preferably contain 1 to 8 double bonds, and the alkenyl carbon chains of 1 to 16 carbons

preferably contain 1 to 5 double bonds. Alkynyl carbon chains of from 2 to 20 carbons preferably contain 1 to 8 triple bonds, and the alkynyl carbon chains of 2 to 16 carbons preferably contain 1 to 5 triple bonds. Exemplary alkyl, alkenyl and alkynyl groups herein include, but are not limited to, methyl, ethyl, propyl, isopropyl, isobutyl, n-butyl, sec-butyl, tert-butyl, isopentyl, neopentyl, tert-pentyl and isohexyl. The alkyl, alkenyl and alkynyl groups, unless otherwise specified, can be optionally substituted, with one or more groups, preferably alkyl group substituents that can be the same or different. As used herein, lower alkyl, lower alkenyl, and lower alkynyl refer to carbon chains having less than about 6 carbons. As used herein, "alk(en)(yn)yl" refers to an alkyl group containing at least one double bond and at least one triple bond.

Please replace the paragraph on page 11 line 26 to page 12 line 12 with the following paragraph:

As used herein, an "aryl group substituent" includes alkyl, cycloalkyl, cycloalkylalkyl, aryl, heteroaryl optionally substituted with 1 or more, preferably 1 to 3, substituents selected from halo, haloalkyl and alkyl, aralkyl, heteroaralkyl, alkenyl containing 1 to 2 double bonds, alkynyl containing 1 to 2 triple bonds, alk(en)(yn)yl groups, halo, pseudohalo, cyano, hydroxy, haloalkyl and polyhaloalkyl, preferably halo lower alkyl, especially trifluoromethyl, formyl, alkylcarbonyl, arylcarbonyl that is optionally substituted with 1 or more, preferably 1 to 3, substituents selected from halo, haloalkyl and alkyl, heteroarylcarbonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocar-bonyl, dialkylaminocarbonyl, arylaminocarbonyl, diarylaminocarbonyl, aralkylaminocarbonyl, alkoxy, aryloxy, perfluoroalkoxy, alkenyloxy, alkynyloxy, arylalkoxy, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, arylaminoalkyl, amino, alkylamino, dialkylamino, arylamino, alkylarylamino, alkylcarbonylamino, arylcarbonylamino, azido, nitro, mercapto, alkylthio, arylthio, perfluoroalkylthio, thiocyano, isothiocyano, alkylsulfinyl, alkylsulfonyl,

arylsulfinyl, arylsulfonyl, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl and arylaminosulfonyl.

Please replace the paragraph on page 12 line 31 to page 13 line 9 with the following paragraph:

As used herein, "heteroaryl" refers to a monocyclic or multicyclic ring system, preferably of about 5 to about 15 members where one or more, more preferably 1 to 3 of the atoms in the ring system is a heteroatom, that is, an element other than carbon, for example, nitrogen, oxygen and sulfur atoms. The heteroaryl can be optionally substituted with one or more, preferably 1 to 3, aryl group substituents. The heteroaryl group can be optionally fused to a benzene ring. Exemplary heteroaryl groups include, for example, furyl, imidazolyl, pyrrolidinyl, pyrimidinyl, tetrazolyl, thienyl, pyridyl, pyrrolyl, N-methylpyrrolyl, quinolinyl and isoquinolinyl, with pyridyl and quinolinyl being preferred.

Please replace the paragraph on page 19 line 29 to page 20 line 2 with the following paragraph:

As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, Biochem. 1972, 11, 942).

Please replace the paragraph on page 24 lines 20 to 27 with the following paragraph:

In another embodiment, the bifunctional hydrazide reagents provided herein form acid cleavable hydrazones. These reagents are advantageous as they can be used to modify biomolecules or carriers such as polymers in a single step. These modified aliphatic hydrazide biomolecules or carriers can subsequently reacted with carbonyl containing biomolecules, drug or other

therapeutic or diagnostic reagent to readily form a hydrazone that can be cleaved following exposure to mild aqueous acid conditions at pH <5.

Please replace the paragraph on page 33 lines 10 to 24 with the following paragraph:

The development of both DNA-based and protein microarrays has led to a revolution in biotechnology. These microarrays are based on immobilization of tens to tens of thousand biomolecules on solid surfaces. Silica based surfaces such as glass slides and silica chips have been the surface of choice to prepare microarrays. The immobilization of biomolecules requires attachment of the biomolecules via covalent or non- covalent, i.e., electrostatic, interactions. Glass slides modified to incorporate amino or aldehyde groups are commercially available (www.arrayit.com, Telechem, Inc, Sunnyvale, CA and www.cel-1.com, Cel Associates, Houston, TX). Protocols to immobilize oligonucleotides or polynucleotides require the use of strong chemical conditions such as sodium borohydride or crosslinking conditions such as photolysis. These methods are inefficient and cause direct modification of the oligonucleotide leading to reduced affinity towards its complementary target.

Please replace the paragraph on page 38 lines 8 to 11 with the following paragraph:

Bifunctional carbazides or thiocarbazides may be prepared by treatment of a hydrazine with phosgene or thiophosgene, respectively, in the presence of base followed by isolation of the iso(thio)cyanate. Addition of hydrazine yields the desired carbazide or thiocarbazide respectively.

Please replace the paragraph on page 38 lines 12 to 15 with the following paragraph:

Bifunctional semicarbazides or thiosemicarbazides may be prepared by treatment of an amine with phosgene or thiophosgene, respectively, in the presence of base followed by isolation of the iso(thio)cyanate. Addition of hydrazine yields the desired semicarbazide or thiosemicarbazide respectively.

Please replace the paragraph on page 41 lines 18 to 25 with the following paragraph:

To a solution of this residue in DMF is added t-butyl carbazate (1.0 equivalent; Aldrich Chemical Company, Milwaukee, WI) in DMF. The reaction mixture is stirred at room temperature for 2 hours. The solvent is removed under reduced pressure and the residue is partitioned between ethyl acetate and 5% aqueous citric acid. The organic phase is washed with brine, dried over magnesium sulfate, filtered and concentrated to give 4-(tert-butoxycarbonylthiosemicarbazidomethyl)cyclohexane carboxylic acid.

Please replace the paragraph on page 41 line 26 to page 42 line 4 with the following paragraph:

This compound (1.0 equivalent) is dissolved in DMF and N-hydroxysuccinimide (1.0 equivalent) is added followed by the dropwise addition of a solution of dicyclohexylcarbodiimide (1.0 equivalents) in DMF. The reaction mixture is stirred at room temperature for 4 hours. The dicyclohexylurea (DCU) precipitate byproduct is removed by filtration and the filtrate is concentrated to dryness. The residue is partitioned between ethyl acetate and 5% aqueous citric acid. The organic phase is washed with brine, dried over magnesium sulfate, filtered and concentrated to give succinimidyl 4-(tert-butoxycarbonylthiosemicarbazido-methyl)cyclohexane carboxylate.

Please replace the paragraph on page 42 lines 21 to 31 with the following paragraph:

This hydrazone (1.0 equivalent) was suspended in DMF and N-hydroxysuccinimide (NHS)(1.0 equivalent) was added and followed by the addition of a solution of DCC (1.0 equivalent) in DMF. The reaction mixture was stirred at room temperature for 16 hours. The heterogeneous reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in a minimum amount of ethyl acetate and hexanes were added to turbidity. A pale yellow precipitate formed that was isolated by filtration to give the desired compound with an approximate yield of 33%. PMR (DMSO-d_6) δ 1.99 (s, 3H), 2.00 (s, 3H), 3.32 (s, 4H), 7.17 (D, 1H), 8.12 (dd, 1H), 8.76 (d, 1H), 10.39 (s, 1H).

Please replace the paragraph on page 43 lines 12 to 18 with the following paragraph:

To a solution of the resulting compound (1 mmol) in THF is added maleic anhydride (1 mmol), the reaction mixture is stirred at room temperature and acetic anhydride (1 mmol) and triethylamine (1 mmol) are added. Following stirring at room temperature for 16 hours, the solvent is removed under reduced pressure, and the residue is chromatographed on silica gel using ethyl acetate as eluant. The fractions containing product are pooled and concentrated.

Please replace the paragraph on page 43 line 26 to page 44 line 3 with the following paragraph:

To a suspension of proline (1 mmol) in THF is added triethylamine (2.5 mmol) followed by the dropwise addition of a solution of thiophosgene (1.1 mmol). The reaction mixture is stirred at ambient temperature for 4 hours followed by cooling the reaction mixture to 0°C and the dropwise addition of a solution of t-butyl carbazate (1.1 mL). The reaction mixture is stirred at 0°C for 1 hour and at room temperature for 2 hours. The solvent is removed under reduced pressure and the residue is

chromatographed on silica gel using methylene chloridemethanol (9/1) as eluant. The fractions containing product are pooled and concentrated.

Please replace the paragraph on page 46 lines 5 to 12 with the following paragraph:

Amino-modified 96 well plates (Costar or Corning) are modified with succinimidyl 4-formylbenzoate (SFB) as follows. A fresh solution of SFB (1.25 mL of 10 mg/mL) in DMSO is prepared. This solution is diluted into phosphate buffered saline (PBS)(0.1 M phosphate, 0.15 M NaCl, pH 7.4: 100 mL). To each well is added 200 μL of the SFB/PBS solution and the wells are incubated at room temperature for 4 hours. The wells are washed three times with PBS/0.5% Tween®. The wells are dried and are ready for protein conjugation.

Please replace the paragraph on page 46 lines 15 to 24 with the following paragraph:

Amino-modified 96 well plates (Costar or Corning) are modified with succinimidyl acetone nicotinic acid hydrazone (SANH) as follows. A fresh solution of SANH (1.25 mL of 10 mg/mL) in DMSO is prepared. This solution is diluted into PBS (0.1 M phospate, 0.15 M NaCl, pH 7.4: 100 mL). To each well was added 200 μL of the SANH/PBS solution and the wells were incubated at room temperature for 4 hours. The wells are washed with water and then treated with 0.1 M acetate, pH 4.7 (200 μL) for 2 hours. The wells were washed three times with PBS/0.5% Tween®. The wells were dried and are ready for conjugation to molecules possessing carbonyl moieties.

Please replace the paragraph on page 46 line 28 to page 47 line 8 with the following paragraph:

A 5 mg/mL solution of bovine serum albumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4 and 2 mM EDTA) (200 μL; 1 mg protein) is prepared. A solution of succinimidyl 4-semicarbazidylbenzoate

hydrochloride (SSCH; 3.5 mg) in DMF (100 µL) is prepared. To the protein solution is added the SSCH/DMF solution (30 equivalents). The reaction mixture is incubated at room temperature for 4 hours. The modified protein is isolated by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with conjugation buffer (3 X 400 µL). The purified protein is quantitated for protein concentration (BCA assay) and for hydrazine modification level by addition of 0.2 mM 2-p-nitrobenzaldehyde in PBS pH 7.4 and measuring the absorbance at 380nm (extinction coefficient 22,600).

Please replace the paragraph on page 47 lines 12 to 25 with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 µL; 1 mg protein) was prepared. A solution of succinimidyl 6-hydrazinonicotinate acetone hydrazone (SANH)(EXAMPLE 2)(2 mg) in DMF (50 µL) is prepared. To the protein solution was added the SANH/DMF solution (15 equivalents). The reaction mixture was incubated at room temperature for 4 hours. The modified protein was isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 µL). The purified protein was quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL) and for hydrazine modification level by incubation of an aliquot of protein in a 0.5 mM 4-nitrobenzaldehyde in 0.1 M MES, 0.9% NaCl, pH 4.7 and measuring the absorbance at 360 nm (molar extinction coefficient 22,000).

Please replace the paragraph on page 48 lines 14 to 22 with the following paragraph:

Aldehyde-modified IgG (EXAMPLE 10) in MES (1 mg; 0.200 µL of a 2.5 mg/mL solution), was added to a solution of hydrazine-modified ovalbumin

(EXAMPLE 11, 1 mL; 0.200 µL of a 5 mg/mL solution) and the reaction mixture was incubated at room temperature for 4 hours. The reaction mixture was analyzed by PAGE gel (coomassie blue development) that demonstrated presence of a high molecular weight product and <5% unreacted aldehyde-modified IgG and <10% unreacted hydrazine-modified ovalbumin. The level of conjugation is quantified by measuring the absorbance at 360 nm.

Please replace the paragraph on page 48 line 25 to page 49 line 3 with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 µL; 1 mg protein) was prepared. A solution of succinimidyl 4-thiosemicarbazidylbenzoate hydrochloride (STBH)(2 mg) in DMF (50 µL) is prepared. To the protein solution was added the STBH/DMF solution (15 equivalents). The reaction mixture was incubated at room temperature for 4 hours. The modified protein was isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 µL). The purified protein was quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL).

Please replace the paragraph on page 49 lines 7 to 11 with the following paragraph:

The thiosemicarbazide protein prepared in EXAMPLE 13 was reacted with aldehyde-modified protein in an identical manner as described for the hydrazine-modified protein in EXAMPLE 12. Analysis by PAGE gel demonstrated similar efficiency as conjugation observed in EXAMPLE 12.

Please replace the paragraph on page 49 lines 14 to 23 with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 µL; 1 mg protein) was prepared. A solution of succinimidyl 4-hydrazidoterephalate hydrochloride (SHTH)(2 mg) in DMF (50 µL) is prepared. To the protein solution was added the SHTH/DMF solution (15 equivalents). The reaction mixture was incubated at room temperature for 4 hours. The modified protein was isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 µL). The purified protein was quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL).

Please replace the title on page 49 lines 25 to 26 with the following title:

Conjugation of a hydrazide-modified protein to an aldehyde modified Protein

Please replace the paragraph on page 49 lines 27 to 31 with the following paragraph:

The hydrazide-modified protein prepared in EXAMPLE 15 was reacted with aldehyde-modified protein in an identical manner as described for the hydrazine-modified protein in EXAMPLE 12. Analysis by PAGE gel demonstrated similar efficiency as conjugation observed in EXAMPLE 12.

Please replace the paragraph on page 51 lines 17 to 22 with the following paragraph:

Periodate-oxidized horseradish peroxidase (Pierce Chemical Co., Rockford, IL) is diluted to the desired concentration and added to 96-3456 well plates that had been modified to possess hydrazino groups as described above in EXAMPLE 9. The antibody solution is allowed to incubate for 2-18 hours followed by removal of the solution and washing with 0.5% Tween® solution (twice) and buffer (twice).

Please replace the paragraph on page 51 line 28 to page 52 line 15 with the following paragraph:

A 25-mer phosphodiester oligonucleotide modified to incorporate a C6-aminolinker (Glen Research amino-C6 amidite) was prepared (5'-NH_2-$(CH_2)_6$-ttt ttt tag cct aac tga tgc cat g-3' (SEQ ID NO.: 1); MW 7791 g/mol, 229.5 OD/μmol; TriLink BioTechnologies, Inc., San Diego, CA). The oligonucleotide was dissolved in conjugation buffer (100 mM phosphate, 150 mM sodium chloride, pH 7.4) to a concentration of 0.92 OD/μL. To a solution of oligonucleotide (64 μL; 2 mg) was added DMF (32 μL). A solution of SANH (EXAMPLE 2; 3.8 mg) in DMF (100 μL) was prepared. An aliquot of the SANH/DMF solution (18.8 μL; 10 equivalents) was added to the oligonucleotide solution and the reaction allowed to incubate at room temperature overnight. The reaction was monitored by C18 RP-HPLC (solution A: 50 mM triethylammonium acetate, solution B: acetonitrile- gradient 0-50% A over 30 min; 50-80% over 10 min; 80-0% over 5 min). The hydrazine-modified oligonucletide was deprotected and purified using a Millipore 5K MWCO ultrafree diafiltration device by diluting the reaction mixture with 100 mM acetate, pH 4.7 and concentrating in the diafiltration device. The retentate was further washed with buffer (2 X 400 μL). The oligonucleotide was quantified by A260 assay and the hydrazine incorporation was determined using the p-nitrobenzaldehyde assay described in EXAMPLE 9.

Please replace the paragraph on page 53 line 21 to page 54 line 8 with the following paragraph:

As shown in FIGURE 9, to a solution of 6-chloronicotinic acid (1 equivalent) in 80% aqueous ethanol is added hydroxylamine (500 equivalents) and the solution is refluxed for 16 hours. The reaction mixture is concentrated to dryness and dissolved in water. The solution cooled in an ice bath and acidified with concentrated hydrochloric acid until a precipitate forms, pH approximately 5.0. The solids are isolated, redissolved in water

and the pH of the solution raised to 7.5 with base. Dioxane (1 volume) is added to the solution followed by the dropwise addition of di-t-butyl dicarbonate (5 equivalents; Aldrich Chemical Co.). The reaction mixture is stirred at room temperature for 4 hours and the dioxane removed on the rotavap. The residue is chromatographed on silica to isolate the desired BOC acid. The acid (1 equivalent) is dissolved in DMF and treated with NHS (1 equivalent) followed by the dropwise addition of DCC (1 equivalent) in DMF. The reaction mixture is stirred at room temperature for 4 hours and the solids removed by filtration and the filtrate concentrated to dryness and resuspended in ethyl acetate. Further precipitate is removed by filtration and the filtrate concentrated to dryness and the desired BOC succinimidyl ester is isolated by silica gel chromatography.

Please replace the paragraph on page 54 lines 20 to 29 with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 µL; 1 mg protein) is prepared. A solution of succinimidyl aminooxyacetate hydrochloride (SAAH)(2 mg) in DMF (50 µL) is prepared. To the protein solution is added the SAAH/DMF solution (15 equivalents). The reaction mixture is incubated at room temperature for 4 hours. The modified protein is isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 µL). The purified protein is quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL).

Please replace the paragraph on page 55 lines 3 to 20 with the following paragraph:

A solution of poly-l-lysine (10 mg; Sigma Chemicals, St. Louis, MO; cat. #P-7890) was dissolved in conjugation buffer, 0.1 M phosphate, 0.15 M NaCl, pH 7.4 (1 mL). A solution of succinimidyl 6-hydrazinonicotinate

acetone hydrazone (SANH; 1.3 mg) was dissolved in DMSO (13 μL). To two poly-l-lysine aliquots (200 μL) were added the SANH/DMSO solution (2.85 μL (10 equivalents) and 5.7 μL (20 equivalents)). The reaction mixtures were vortexed and incubated at room temperature for 2 hours. The modified polymer was isolated by gel filtration on a NAP-25 column (Pharmacia) pre-equilibrated with 0.1 M MES, 0.9% NaCl, pH 4.7 buffer. Fractions (1 mL) were collected and analyzed by UV (A260). Fractions containing UV active product were combined to yield the desired product. The product was analyzed colorimetrically for hydrazine content by dissolving an aliquot (2 μL) in a 0.5 mM solution of p-nitrobenzaldehyde (98 μL) and incubating at 37°C for 1 hour followed by taking A390 readings (extinction coefficient 22000). The HyNic:poly-l-lysine polymer was used directly in the conjugation step. The amine/hydrazine content was determined using the TNBSA assay (trinitrobenzenesulfonic acid; Pierce Chemical, Inc., Rockville, IL).

Please replace the paragraph on page 55 lines 24 to 30 with the following paragraph:

A solution of a bacterial polysaccharide that possesses unsaturation in its lipids (from ATCC; 10 mg/mL) in water is treated with 10 mM sodium periodate (1/10 volume to make the solution 1 mM in periodate) and incubated at room temperature for 30 minutes. The reaction mixture is passed through a sephadex G-25 column pre-equilibrated with water to remove small molecule impurities. The polysaccharide fractions are combined and concentrated to 5 mg/mL.

Applicant submits that to the best of his knowledge these amendments do not add new matter to the specification.